Posters

Protein Structures

246-Pos Board B1
Structure and Function of Two Putative Virulence Factors from Francisella Tularensis
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F. tularensis is a poorly understood category A priority pathogen and the etiological agent of Tularemia, or rabbit fever. Previous work by coworkers at LLNL identified seven putative virulence factor proteins, for which most have weak homology to proteins of known function. These seven proteins presumably are secreted host effector proteins, as they are important for inducing an encysted morphology in F. tularensis infected amoeba. Subsequent genetic studies have further implicated some of these ORFs as having a role in infection of human cells in culture. We have applied a structural-functional genomics approach in an attempt to elucidate the function of these rapid encystment proteins (REPs). Here we present the crystal structures of REP24 (24 kD) and REP34 (34 kD) and report on their putative function. REP24 appears to be a cysteine protease with a novel arrangement of the active-site catalytic triad, and we show that it binds to the cysteine protease inhibitor, JPM-565, both in vitro and in crystallo. REP34 By structural homology and, REP34 appears to be a Zn-dependent carboxypeptidase that possesses a novel fold that deviates from related funnelins, resulting in a rearranged active and substrate-recognition sites. We show the definitive presence of a Zn by anomalous diffraction, and catalytic activity of the enzyme using hippuryl-L-Arg. We believe that these structures provide potential starting points for rational design of new therapeutics against tularemia and, combined with further biochemical evidence, will provide important insights into the molecular mechanisms of F. tularensis infection.

247-Pos Board B2
Crystal Structure of an Inactive Variant of the Vibrio Cholerae Quorum-Sensing Master Regulator HapR
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Virulence and biofilm formation are among a number of processes in Vibrio cholerae controlled by the quorum-sensing master regulator HapR. The crystal structure of HapR revealed it to be a member of the TetR family of transcription factors with an N terminal helix-turn-helix DNA binding domain and a C terminal dimerization domain. An inactive variant of HapR was found to contain a glycine to aspartic acid substitution at position 39 within the N-terminal hinge region. Size exclusion chromatography and circular dichroism revealed no significant structural differences between the normal and variant HapR. However, structural reconstruction using small/wide angle x ray scattering data suggested that the arrangement of the DNA binding domains of the variant HapR was altered. To gain further insight into the functional role of the N terminal hinge region of HapR and the structural consequences of a substitution of aspartic acid for glycine at position 39, we have solved the crystal structure of the variant HapR to 2.3 Å.

248-Pos Board B3
X-Ray Structure Determination of the First Insect Skeletal Muscle Myosin II
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Skeletal muscle myosin class II proteins are molecular machines that convert the chemical energy derived from the hydrolysis of ATP into mechanical work used to power muscle contraction. The three-dimensional structure of the truncated head from an embryonic body wall myosin isoform (EMB) of the common fruit fly, Drosophila melanogaster, is reported at 2.2 Å resolution. The histidine-tagged recombinant protein was expressed in and purified from the indirect flight muscles of an engineered fly line (Caldwell et al., Methods, 2012). The purified histidine-tagged EMB myosin retains ATPase activity similar to that of the untagged EMB isoform. EMB myosin subfragment 1 (S1), contains the myosin heavy chain motor domain (MD) and the essential light chain (ELC). Crystals of the complex belong to space group P212121 with the unit cell parameters of a = 108.5 Å, b = 148.5 Å, and c = 148.7 Å. Two copies of the molecule were resolved in the asymmetric unit and these have slight conformational differences. Interestingly, a number of the domains encoded by alternative exons are in contact between the two molecules, suggesting potential isoform-specific interactions that may be relevant in vivo. While the nucleotide-binding site had some ligand electron density, the ligand was not resolved. The enzymatic conformational state of the myosin cross-bridge cycle was determined as post-rigor by comparisons with known myosin structures. Amino acid residue orientations are currently being compared between EMB S1 and other myosins, particularly in the alternatively-encoded regions, to gain insights into structure-function relationships that define isoform-specificity.

249-Pos Board B4
The Catalytic Subunit of the SWR1 Remodeler has a Histone Chaperone Role for the H2A.Z-H2B Dimer
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Histone variant H2A.Z-containing nucleosomes exist at most eukaryotic promoters and play important roles in gene transcription and genome stability. The multi-subunit nucleosome-remodeling enzyme complex SWR1, conserved from yeast to mammals, catalyzes the ATP-dependent replacement of histone H2A in canonical nucleosomes with H2A.Z. The over-incorporation of H2A.Z in chromatin, however, is associated with breast cancer, and therefore H2A.Z and p400/SRCAP are targets for cancer therapy. How SWR1 catalyzes the replacement reaction is largely unknown. Here we determined the crystal structure of the region (599-627) of the catalytic subunit Swr1, termed Swr1-Z domain, N-terminal to the ATPase domain, in complex with the H2A.Z-H2B dimer at 1.78 Å resolution. The Swr1-Z domain forms a 3(10) helix and an irregular chain, separated by an 8-residue disordered loop. Swr1-Z forms hydrophobic interactions with the a3 and aC helices of H2A.Z, and also with the a1 and a2 helices of H2B. Importantly, a conserved LxxLF motif in the Swr1-Z specifically recognizes the αC helix of H2A.Z, which leads to an extension of two helical turns of the aC helix in H2A.Z. Additionally, the Asp-rich region in Swr1-Z forms electrostatic interactions with Arg85 in the L2 loop, and with Arg89 in the a3 helix of H2A.Z, and with Lys60 in the a2 helix of H2B, which makes the complex more stable. Furthermore, in vitro studies show that Swr1-Z domain can deliver the H2A.Z-H2B dimer to the DNA-(H3-H4)2 tetrasome to form the nucleosome by a histone chaperone mechanism, and in vivo studies indicate that Swr1-Z has an important functional role. Our results provide the first structural insights into how Swr1 recognizes H2AZ-H2B, and help identify similar binding regions between human H2AZ-H2B and p400/SRCAP, paving the road for structure-based cancer drug design.

250-Pos Board B5
Structure and Function of the Genomically-Encoded Fosfomycin Resistance Enzyme, FosB, from Staphylococcus Aureus
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The Gram-positive pathogen *Staphylococcus aureus* is a leading cause of global morbidity and mortality. Like many multi-drug resistant organisms, *S. aureus* contains antibiotic modifying enzymes that facilitate resistance to a multitude of antimicrobial compounds. FosB is a Mn^{2+}-dependent fosfomycin-inactivating enzyme found in *S. aureus* that catalyzes nucleophillic addition of either L-cysteine (L-Cys) or bacillithiol (BSH) to the antibiotic resulting in a modified compound with no bacteriacidal properties. The three-dimensional x-ray crystal structure of $FosB^{Sa}$ has been determined to a resolution of 1.15 Å. Co-crystallization of $FosB^{Sa}$ with either L-cys or BSH results in an unnatural disulfide bond between the exogenous thiol and the active site Cys9 of the enzyme. Two crystals of $FosB^{Sa}$ contain Zn^{2+} in the active site, but subsequent kinetic analyses indicated that the enzyme is inhibited by Zn^{2+} for L-Cys transferase activity and only marginally active for BSH transferase activity. Fosfomycin-treated disk diffusion assays involving *S. aureus* Newman and the USA300 methicillin-resistant *S. aureus* (MRSA) demonstrate a marked increase in sensitivity of the organism to the antibiotic in either the BSH or FosB null strains, indicating that both are required for survival of the organism when treated with fosfomycin. This work identifies FosB as the sole fosfomycin-modifying pathway of *S. aureus* and establishes the enzyme as a potential therapeutic target for increased efficacy of the antibiotic against the pathogen.